AD NUMBER

AD835844

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Critical Technology; 23 NOV 1964. Other requests shall be referred to Army Biological Laboratory, Attn: Technical Release Branch [TID], Fort Detrick, MD 21701.

AUTHORITY

SMUFD, d/a ltr, 14 Feb 1972

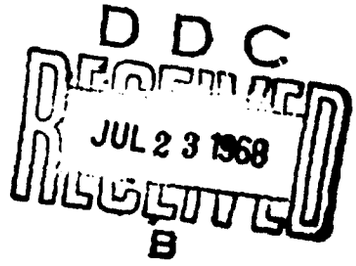
AD 635844

TRANSLATION NO.

DATE:

DDC AVAILABILITY NOTICE

Reproduction of this publication in whole or in part is prohibited. However, DDC is authorized to reproduce the publication for United States Government purposes.



STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of _____

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Tech Release Br. TID

BIOCHEMICAL STUDIES ON THE RICE BLAST DISEASE (PART 10) BIOCHEMICAL CLASSIFICATION OF PIRICULARIA ORYZAE CAVARA (NO 4)

- Japan -

[Following is a translation of an article by Otsuka, H. et al., in the Japanese-language journal, Agricultural Chem. Soc. Japan Journal, Vol 31, 1957, pages 890-893.]

In our previous reports Part 10 (No 2) (1) and Part 10 (No 3) (2), (we have examined 45 P.o.c. stock-cultures with regard to the extent of their consumption of carbon and nitrogen sources and demonstrated the existence of stock-culture differences, which in turn made possible the classification of Piricularia oryzae Cavara (hereafter referred to as P.o.c.). We have further noted that such a scheme of classification was closely related to that of Agricultural Res. Ctr. based on the difference in the capacity of different kinds of wet rice to resist P.o.c.

F. W. Leaver (3) showed biotine and thiamine-HCl to be indispensable ingredients for the growth of P.o.c., and the optimal density of biotine was shown to lie between 0.001 - 0.01 ug/ml. In the synthetic culture medium, biotine was also shown independently by Tanaka and others (4) to be an indispensable ingredient for P.o.c. growth.

In a similar vein, Otani (5) experimented with the addition to the synthetic cultivating soil of biotine extracted from synthetic compounds and beef liver and intestines, and an optimal density was found to be 2.3 - 3.0 mγ/ml. It was also noted that with the majority of P.o.c. biotine turned out to be indispensable, and that the few types

not needing biotine were rather exceptional cases.

We have examined the propagation of 45 P.o.c. stock-cultures in a biotine-free culture medium. Out of the 45 stocks, two stocks showed a fair degree of propagation, and another four revealed the meager signs of some propagation. On the two well-propagating stocks we have performed quantitative analysis by means of Lactobacillus arabinosus bioassay; the interesting results of this are reported in the present paper.

Experiment

1) P.o.c. used in the experiment: the 45 stock-cultures used in our report Pt. 10 (No 1).

2) Prior cultivation: As described in our report Pt. 10 (No 1).

3) Culture medium: the composition of the culture medium is as shown in Table 1. All the components of Table 1 except glucose and thiamine were thoroughly dissolved, and this was sterilized for 15 minutes at 15 pounds of pressure. Glucose and thiamine were then added and dissolved, and this was then transferred, 2 ml, each into test tubes of approximately 17 mm in diameter. Before serving as culture media, they were sterilized for 10 minutes at 10 pounds.

Table 1

Composition of culture medium for testing biotine

① グルコース	20 g
KNO_3	3 g
K_2HPO_4	1.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$CaCl_2$	0.1 g
$FeSO_4 \cdot 7H_2O$	0.0075 g
$MnSO_4 \cdot 7H_2O$	0.002 g
$CuSO_4 \cdot 5H_2O$	0.006 g
$ZnCl_2$	0.075 g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.009 g
② チアミン	1 mg
③ 蒸留水を加えて 1l とす (pH 7.0)	

[Legend:] 1) Glucose; 2) Thiamine; 3) Add distilled water to make 1l (Ph. 7.0).

4) Determination of propagation: as in our report Pt. 10 (No 3), each stock-culture was inoculated and cultivated for 14 days at 25°, then washed with water and dried, until it reached a stable weight, at 100° (average 4-6 hours). Extent of propagation was determined by comparing the weights of dried bacteria. The results are shown in Table 2.

Table 2

Bacteria weights on the biotine-free culture medium

②菌体	①菌体重量×200 ③対照*	①菌体重量×200 ④ビオチン欠培地	②菌株	①菌体重量×200 ③対照*	①菌体重量×200 ④ビオチン欠培地	②菌株	①菌体重量×200 ③対照*	①菌体重量×200 ④ビオチン欠培地
5414	0.05	0.05	5333	0.30	[illegible]	5523	0.20	0.20
5418	0.10	〃	5404	0.05	[illegible]	5521	0.05	0.05
3	0.05	〃	5420	〃	[illegible]	5525	〃	〃
No. 1	〃	1.00**	5424	0.20	0.40	5526		0.03
No. 2	〃	1.20**	5425	0.05	0.05	5527		〃
No.11 F8h	0.03	0.03	5415	0.10	0.30	5528		0.05
No. 11 h	〃	0.05	5514	0.05	[illegible]	5529	〃	0.40
No. 188 h	0.10	〃	5515	0.20	[illegible]	5532	0.10	0.10
P_2	0.15	〃	5516	0.05	0.10	5533	0.20	0.30
A 25	0.05	〃	5517	〃	0.30	5534	〃	0.10
A 36	〃	〃	5518	0.20	0.05	5535	〃	0.05
5309	0.10	〃	5519	0.10	〃	5536	0.05	〃
5311	0.05	〃	5520	〃	[illegible]	5537	〃	〃
5327	0.20	0.70	5521	0.10	[illegible]	5539	〃	〃
5330	0.10	0.10	5522	0.05	[illegible]	5540	0.10	0.10

⑤ * 対照とは第1表組成より KNO_3 3g をのぞきビオチン [illegible] 添加せる[illegible]繁殖せしめた菌体重量を示す.

⑥ ** 第1表組成にビオチン 5γ/l 添加した完全培地に[illegible] 値は No.1 : 2.00, No.2 は 3.60 である.

[Legend:] 1) Bacteria weight x 200; 2) Stock-culture; 3) Control; 4) Biotine-free culture medium; 5) By control is meant the bacteria weight cultivated in the medium which had biotine 5γ/l substituted for KNO_3 3g from the composition of Table 1; 6) Bacteria weights in the complete culture medium with biotine 5γ/l added to the composition of Table 1 to were 2.00 for No 1 and 3.60 for No 2.

5) Measurement of biotine quantity: Confirmation of biotine absence in the cultivating soil and measurement of biotine quantity were by the method of bioassay which utilized Lactobacillus arabinosus ATCC 8014. The bioassay technique was first cultivating for 72 hours at 37°, and then titrating 2 ml of cultivating solution with 1/20 n NaOH. The composition of bioassay culture medium is as shown in Table 3.

Table 3

Composition of biotine bioassay culture medium

1) グルコース	4 g	16) L-チロシン	20 mg
2) Na-アセテート	1 g	17) DL-バリン	40 mg
3) DL-アラニン	40 mg	18) アデニン・H_2SO_4	2 mg
4) アスパラギン	20 mg	19) グアニン・HCl	2 mg
5) L-アスパラギン酸	80 mg	20) ウラシル	2 mg
6) L-アルギニン-HCl	40 mg	21) キサンチン	2 mg
7) L-シスチン	20 mg	22) チアミン	200 γ
8) L-グルタミン酸	100 mg	23) リボフラビン	200 γ
9) L-イソロイシン	20 mg	24) ピリドキシン-HCl	200 γ
10) L-ロイシン	20 mg	25) ピリドキサール-HCl	200 γ
11) L-リジン-HCl	40 mg	Ca-Pantothenate	200 γ
12) DL-メチオニン	20 mg	26) ニコチン酸	200 γ
13) L-フェニルアラニン	40 mg	p-Aminobenzoic acid	60 γ
14) DL-スレオニン	40 mg	Folic acid	2 γ
15) DL-トリプトファン	20 mg		

㉗ A液 2 ml (KH_2PO_4 0.2 g, K_2HPO_4 0.2 g)
B液 2 ml ($MgSO_4 \cdot 7H_2O$ 0.08 g, NaCl 0.004 g, $FeSO_4 \cdot 7H_2O$ 0.001 g, $MnSO_4 \cdot 7H_2O$ 0.004 g)
㉘ 蒸留水を加え 100 ml とす (pH 6.8)

[Legend:] 1) Glucose; 2) Na-acetate; 3) DL-alanine; 4) Asparagine; 5) L-aspartic acid; 6) L-arginine-HCl; 7) L-cystine; 8) L-glutamic acid; 9) L-isoleucine; 10) L-leucine; 11) L-lysine-HCl; 12) DL-methionine; 13) L-phenyoalanine; 14) DL-threonine; 15) DL-tryptophane; 16) L-tyrosine; 17) DL-barine; 18) Adenine-; 19) Guanine-; 20) Uracil; 21) Xthantine; 22) Thiamine; 23) Riboflavin; 24) Pyridoxine-; 25) Pyridoxal-; 26) Nicotinic acid; 27) solution; 28) Add distilled water to make 100 ml (ph. 6.8).

The standard curve obtained by this method is given in Fig. 1.

Since biotine is known to exist in cotton (6), the biotine quantity in the culture medium of Table 1 that used cotton plug was quantitatively determined, and biotine was shown to be non-existent.

No. 1 and No 2 were cultivated for 14 days at 25° in the culture medium as specified in Table 1. Buffer solution (selenic acid 1/15 M phosphate, pH 6.98) was added to the cultivating solution and water-washed bacteria, ground and left for 24 hours at 40°, and then subjected to biotine quantification. The results are shown in Table 4.

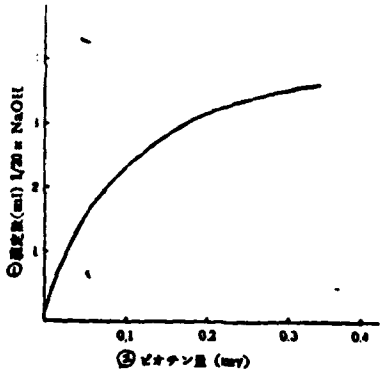


Fig. 1. Reference curve for biotine

[Legend:] 1) titration amount; 2) biotine quantity.

Table 4

Biotine contained in the cultivating solution and in the bacteria as well

① 菌株	②培養液のビオチン量 (mγ/ml)	③乾燥菌体のビオチン量 (mγ/g)
No. 1	0.0	51.5
No. 2	0.0	135.8

[Legend:] 1) Stock-culture; 2) Biotine quantity in the cultivating solution; 3) Biotine quantity contained in the dried bacteria.

Discussion

Results of the present study indicate that the majority of P.o.c. stock-cultures do not propagate in the biotine-free synthetic culture medium, which is in agreement with the findings of Leaver, Otani and Tanaka. However, No 1 and No 2 are somewhat exceptional. Compared to the growth on the complete synthetic culture medium, they have shown increase of 1/2 - 1/3 of bacterial weight.

Compared to the control, other stocks such as 5327, 5517, 5529 showed some sign of propagation.

Thus, one could divide P.o.c. into those that propagate in the biotine-free synthetic culture medium and others that do not, which seems to underline the fact that the amount of biotine demand varies depending on the P.o.c. stock-culture. Stocks such as No 1 and No 2 do notneed biotine at all.

In order to pursue this matter further, we have cultivated biotine-non-dependent No 1 and No 2 on biotine-free soil, and determined the amount of biotine in the cultivating solution and in the self-digested substance in bacteria by the bioassay technique that utilizes Lactobacillus arabinosus ATCC 8014. From the results presented in Table 4, we note that biotine is completely absent in the cultivating solution for both No 1 and No 2, while 51.5 mγ/g for No 1 and 135.8 mγ/g for No 2 respectively were found in the bacteria bodies. This finding seems to suggest that 0.13 mγ and 0.41 mγ biotine were formed per 1 ml of cultivating solution for No 1 and No 2 respectively (0.13 mγ was deduced from obtaining 0.01 g of dried bacteria from 4 ml of biotine-free cultivating soil).

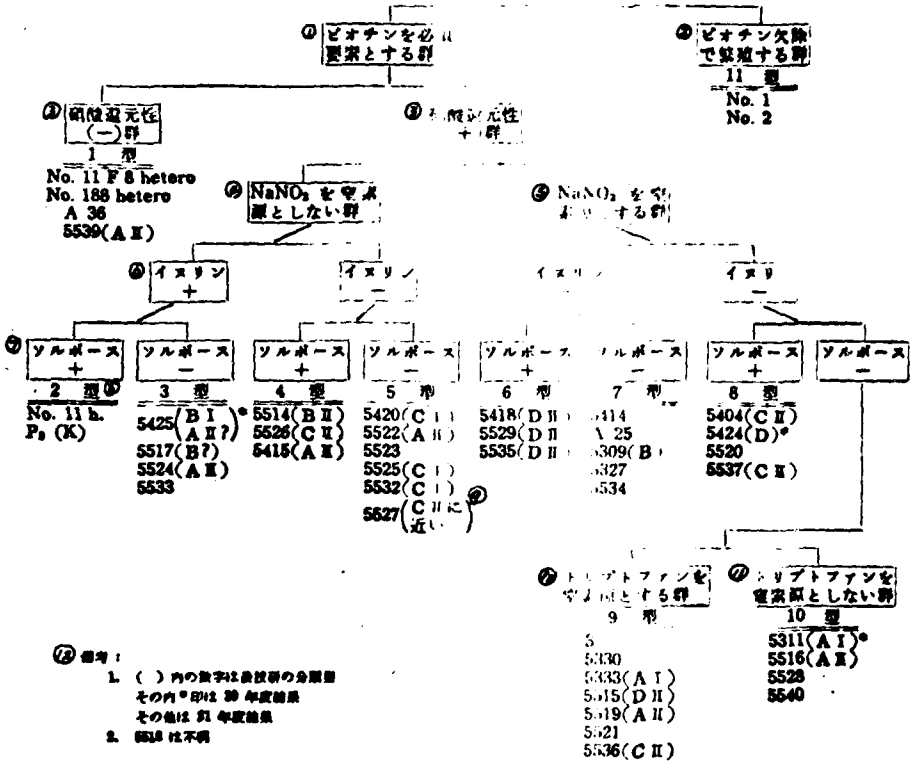
This figure, by comparison, is far less than the optimal density of 0.01 - 0.02 μg/ml by Leaver and 2.3 - 3.0 mγ/ml by Otani. According to the existing literature (7), the within-tissue biotine quantity is far less in the case of acid decomposition than in the case of self-digestion, which suggests the needs of future study. At any rate, No 1 and No 2 not only need little biotine in the cultivating base, but also have small capacity for biotine formation.

We regarded the differences among stocks with regard to the extent of biotine dependency to be a meaningful key for classificatory purpose and have revised the table of classification of our report Pt. 10 (3) as shown in Table 5.

As Table 5 is self-explanatory, we have taken into consideration the fact of both No 1 and No using $NaNO_2$ as nitrogen source, and have divided the 45 stock-cultures into 11 groups, thus separating out No 1 and No 2 from the rest. In all biochemical properties other than the biotine difference, type 11 is akin to the types 6-10. Thus, the only feature differentiating the present scheme of classification from that in our report Pt. 10 (No 3) is the addition of the 11th subgroup.

Table 5

The Table of P.o.c. classification



[Legend:] 1) Biotine-dependent group; 2) Biotine-non dependent group; 3) Nitric acid reducability; 4) $NaNO_2$ not used as nitrogen source; 5) $NaNO_2$ used as nitrogen source; 6) Inulin; 7) Sorbese; 8) type; 9) close to C 11; 10) Tryptophane used as nitrogen source; 11) Tryptophane not used as nitrogen source; 12) Remarks: 1 -- The numbers within the bracket ( ) are for the typology of Agr. Res. Ctr. Those with asterisks are the results of 1955. The rests are the result of 1956; 2 -- 5518 is ambiguous.

Summary

Following results were obtained by cultivating 45 stock-cultures of P.o.c. in the biotine-free base.

1) No 1 and No 2 show a fair degree of propagation, while four other stocks propagate a little and the remaining 39 stocks did not propagate at all.

2) Bioassay results on the biotine quantity in the cultivating solution and in the self-digested substance of washed-bacteria of No 1 and No 2, which were first cultivated in the biotine-free base, revealed a minute degree of biotine formation.

3) The 45 P.o.c. stock-cultures were classified into 11 groups depending upon the extent of sugar and nitrogen consumption and the nitric acid reducibility.

References

1. Otsuka, H., et al; This journal, 31, 794 (1957).

2. Otsuka, H., et al; This journal, 31, 886 (1957).

3. Leaver, F. W., Leal, J., Brewer, C. R., J. Bact., 54, 401 (1947).

4. Tanaka, S., Kagaku Kenkyujo Hokoku ("Bulletin of Chem. Res. Inst.") 20, 1 (1950). Nogyo Gijutsu ("Agr. Technology") 6, 1 (1951). Nika ("Japanese Chemistry") 72, 231, 285 (1951). Nishokubyo ("Jap. J. of Plant Pathology") 109, 15, (1951).

5. Nishokubyo ("Jap. J. of Plant Pathology"), 17 (1), 9 (1953).

6. Wada, S., Yakgakuzasshi ("Pharmaceutical Magazine) 74, 668 (1954).

7. Tanaka, S. et. al., Kagaku No Ryoiki ("Realms of Chemistry") 6 (11), 633 (1952).

- END -